THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Preservation Effectiveness of Hydrogen Peroxide and Honey on Raw and Pasteurized Milk

Samson, K. S.
Assistant Lecturer, Department of Animal Production,
University of Agriculture Makurdi, Makurdi, Benue State, Nigeria
Attah, S.
Professor, Department of Animal Production,
University of Agriculture Makurdi, Makurdi, Benue State, Nigeria
Shaahu, D. T.
Senior Lecturer, Department of Animal Production,
University of Agriculture Makurdi, Makurdi, Benue State, Nigeria

Abstract:
A study on the preservation effectiveness of hydrogen per oxide and honey on raw and pasteurized milk for 30 hours was carried out. Fresh milk samples collected from Makurdi were divided in to 2 portions. One portion was subjected to pasteurization there after sub-divided in to three. One was treated with 0.03% honey, the second with 0.03% H_20_2 and the third was kept as pasteurized milk (the control). The second portion of the fresh milk was kept raw but treated with same quantity of the preservatives as in the first. Some chemical parameters namely; protein, fat, carbohydrate moisture and ash were analyzed at the 1^{st} hour of collection and after 30hours post treatment. For all the parameters the values at 30hours post treatment decreased significantly across treatments, only the moisture of samples treated with H_2O_2 at 30hours was observed to have increased. The pH was determined by the use of a pH meter while the microbial load was determined using 1ml of milk samples serially diluted (10^1 to 10^5) in sterile water and 200µl of samples were plated unto nutrient agar plates and incubated at 37°C for 24hrs. Some physical parameters such as texture and flavor were measured using visual appraisal for all samples just before the preservation and then at 6 hour interval for 30hours. From the result of the physical and chemical test, it was observed that keeping quality of milk sample especially pasteurized milk treated with hydrogen peroxide increased significantly compared to milk treated with honey and that of the control for all samples. The result of the physical and chemical parameters explains deterioration before the end of the experiment in both samples. It was concluded that 0.03% hydrogen peroxide is enough to preserve milk for 12 hours.

Keywords: *Hydrogen peroxide (H_2O_2), Honey, Raw milk, pasteurized.*

1. Introduction

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the method of production it is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality (Rogelj, 2003). Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al.*, 2008). The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (Rogelj, 2003). Milk contains carbohydrate in form of lactose, fat, vitamins and chloride, inorganic phosphate and citrate (Komorowski, 1992). Dairy product is essential part of the Nigerian food, therefore attention ought to be paid in to hygienic aspect of handling and distributing of such foods. This study was under taken to ascertain the effect of treating both raw and pasteurized milk with honey and hydrogen per oxide.

2. Materials and Methods

The study was carried out at the Microbiology Laboratory of the Veterinary Teaching Hospital University of Agriculture Makurdi, Benue state of Nigeria. Makurdi is located in the Southern Guinea Savanna zone (Latitude 7°43′N and longitude 8°3′E). The area is warm with a minimum temperature of 24.20ºC and maximum temperature of 36.33ºC. The rainfall is between 508 and 1016mm and the relative humidity is between 39.50±2.20 and 64.00±4.8% (TAC,2009)

2.1. Sample Collection/Processing

Fresh milk samples (2000mls) were taken from a pool of milk collected from white Fulani herds and samples were divided into two parts to assess the storability of pasteurized (PM) and raw milk(RM), both of which serve as control, and a portion of each was treated as follows

a. Food grade hydrogen peroxide was added at 0.03% to obtain PM+H_2O_2; RM+ H_2O_2

b. Undiluted honey was added at 0.03% to obtain PM+Honey; RM+ Honey

There were thus 6 test samples, namely PM, PM + H_20_2, PM + Honey, RM, RM +H_20_2, RM + Honey

2.2. Experimental Treatment/ Procedures

The fresh milk samples after thorough mixing were divided into 2 equal parts of 1000ml each. One of the 2 parts was pasteurized (at 95°C and held for 30minutes followed by immediate cooling) and further divided into 3of 333ml each One part of the pasteurized milk was treated with 0.03% hydrogen peroxide, following the procedure of Saha *et al.* (2003), another part with 0.03% honey and the third part was kept as pasteurized milk which served as control. The unpasteurized milk sample was equally divided in to 3, 1 part was kept untreated which served as the control, another part was treated with the same quantity of hydrogen peroxide and the last part was treated with the same quantity of honey. The physical (texture and flavour), chemical (Hydrogen ion concentration which is the pH) qualities, proximate analysis and microbial load of milk were determined before and after adding the preservatives.

3. Laboratory Analyses

3.1. Microbial Load

The determination of microbial load was done using 1ml of milk samples serially diluted (10^1 to 10^5) in sterile water and 200µl of samples were plated unto nutrient agar plates and incubated at 37°C for 24hrs. The numbers of colonies were counted afterwards using a Standard Counter. Sampling was carried out 3 hourly for 30 hours post treatment for all the samples.

3.2. Chemical Parameters

The samples were analyzed for protein, fat, carbohydrate, moisture and ash. The fat was estimated by the Roese-Gottlieb method (Supplee and Bellis, (2014), milk protein (N x 6.38) was determined using the semi-micro Kjeldahl and Markhams Distillation Apparatus and the ash content was obtained by drying and ashing a weighed milk sample (10ml) to a constant weight as 550 °C for 48 hours, while the moisture content was determined based on the principle of drying to constant weight has described by Osborne and Voogt (1978). The pH was determined by the use of a pH meter (WPA CD6). Determinations were done at the onset of treatments and at the end of 30 hours.

3.3. Physical Parameters

Sensory qualities of milk were evaluated by a jury of 5 panelists to determine the texture and flavour at 6 hourly intervals following the procedure of Meilgaard *et al.* (1999). The panelists tasted the samples and were asked to keep the milk in their mouth for 12 seconds before scoring. The milk samples were presented in random order. Water was used for rinsing mouth between samples (International Dairy Federation, 2002).

3.4. Statistical Analysis

Microsoft Excel spread sheet (2006) was employed for raw data entry. Transformation of microbial count was done using average dilution x 1/dilution factor x $^1/_{0.1}$ before the analysis, The data obtained were subjected to statistical analysis using SPSS (2010) and means that were significantly different were separated using least significant difference (LSD) as contained in SPSS (2010) for Windows (version 16). For all analysis, 95 % CF (confident factor) and P(probability)-value<0.05 was set for statistical significance of an estimate.

4. Results and Discussion

Average chemical composition of raw milk (RM) and pasteurized (PM) treated with honey (H) and hydrogen peroxide (H_20_2) in Table 1, did not show much difference in the milk protein content. This is in agreement with the report given by Seskena and Jankerita (2007) who mentioned that the effect of the raw milk treated with hydrogen peroxide at 0.03% did not have any noticeable effect on milk protein content. From this study, it was also observed that carbohydrate content of milk treated with honey was higher than that of the control and the milk treated with H_20_2. This is probably because honey is composed primarily of the sugars of glucose and fructose which are the end products of carbohydrate digestion. H_20_2 is not a stable compound, it decomposes in to water and oxygen. This explains the higher moisture content of milk treated with H_20_2. It is assumed that when heat such as the heat of pasteurization is applied to a liquid it loses moisture, this justifies the reason why the moisture content of the pasteurized milk was lower than that of the fresh milk. The proximate composition after 30 hours post treatment showed a decrease in all the parameters. It is possible that the microorganisms present in the milk made use of the nutrient present for their metabolism.

Parameters	Time(hr)	RM	RM+H	RM+H_2O_2	PM	PM+H	PM+ H_2O_2
Crude Protein	1^{ST}	3.15	3.20	3.18	3.22	3.13	3.19
	30^{Th}	2.45	2.81	3.00	3.05	2.91	3.00
Moisture	1^{ST}	85.8	85.3	86.5	84.5	84.8	86.4
	30^{Th}	85.4	83.1	87.0	85.2	84.8	89.3
Fat	1^{ST}	4.05	4.32	3.99	3.85	4.13	4.05
	30^{Th}	4.00	4.32	3.98	3.81	4.00	4.01
Carbohydrate	1^{ST}	4.78	6.33	4.59	4.79	5.56	4.79
	30^{Th}	2.18	5.00	4.01	4.61	5.50	4.59
Ash	1^{ST}	0.77	1.00	0.70	0.68	1.11	0.79
	30^{Th}	0.70	0.70	0.69	0.64	1.00	0.73

Table 1: Proximate Composition of Raw and Pasteurized Milk Treated with Honey and H_2O_2
RM= raw milk, PM =pasteurized milk, hr= hour, H= honey

From table 2, there was a rapid multiplication of microbial load especially in the raw milk. This however reduced progressively in RM+H, PM, and PM+H, RM+H_20_2 and PM+ H_20_2. It is a well-known fact that hydrogen peroxide inhibits the growth of bacterial population and this could be the reason why the microbial load of the sample treated with H_20_2 remain minimal at the end of the experiment. This confirms the findings of Saha ,*et al* (2003) who noted that H_20_2 effectively inhibit the growth of bacteria in raw milk under rural conditions , he also reported that the addition of 0.03 -0.04% H_20_2 to raw milk is enough to preserve milk for up to 22-24hours. Similarly, Nzeakor and Handi (2000) reported that honey preserves bovine milk by reducing the survival of bacteria. This is in line with the findings of the present study, in that the milk treated with honey has lesser microbial load compared to raw milk which served as control. Griffiths *et al.* (1998) observed a correlation between storage time and psychotropic bacteria of both raw and pasteurized milk, this is in agreement with the findings in this study in that with increase storage time, there was an increase in the microbial count. Raw milk total microbial load should not exceed 1.0 x105 cfu/ml according to Turkish food code x(CNO.2009/14) and commission regulation (IC, NO 1662/2006). In this study the microbial load of fresh milk was 3.7x 10^3cfu/ml higher than the limit stated by these two agencies. The pasteurized milk used in this study meets the requirement of the FDA, (2009) which states that pasteurized Grade A milk is required to have less than 2.0 X 10^4 cfu/ml total count. Rodríguez-Alcalá *et al.*, (2009) mentioned that microorganisms multiply rapidly when the milk is kept at ambient temperatures after milking. The lactic acid produced causes the natural Souring of milk, he also stated that the primary source of these bacteria include environment, air, dust, dirty equipment and operators. How soon the milk turns sour depends on the degree of contamination and on the temperature of the milk

Time (hour)	RM	RM+ Honey	RM+H_20_2	PM	PM+Honey	PM+ H_20_2
0	$3.7x10^{2c}$	$3.5x10^{2d}$	$3.0x10^2$	TS	TS	TS
3	$4.8x10^{2c}$	$4.0x10^{2d}$	$3.8x10^2$	TS	TS	TS
6	$6.5x10^{2ab}$	$6.2x10^{2c}$	$5.2x10^2$	TS	TS	TS
9	$8.4x10^{2b}$	$8.0x10^{2b}$	$5.3x10^2$	TS	TS	TS
12	$8.8x10^{2b}$	$8.4x10^{2b}$	$5.2x10^2$	$2.2x10^2$	$2.8x10^2$	TS
15	$8.3x10^{2a}$	$8.6x10^{2a}$	$6.5x10^2$	$5.7x10^2$	$4.2x10^2$	TS
18	TNC	TNC	$6.5x10^2$	$7.4x10^2$	$6.9x10^2$	TS
21	TNC	TNC	$8.0x10^2$	$2.0x10^1$	$8.1x10^2$	$2.0x10^2$
24	TNC	TNC	$8.2x10^2$	TNC	$8.2x10^2$	$3.1x10^2$
27	TNC	TNC	$8.1x10^2$	TNC	$8.0x10^2$	$3.9x10^2$
30	TNC	TNC	TNC	TNC	TNC	$5.7x10^2$

Abcd Means with different superscript are significantly different ($p<0.05$). RM= Raw milk, PM=Pasteurized milk, TS= Too scanty, TNC= Too numerous to count

Table 2: Effect of Storage Time on Microbial load of Raw and Treated milk

The flavor quality and flavor score of honey and hydrogen peroxide treated milk and untreated milk samples presented in Table 3 showed RM, RM+H, RM+ H_20_2, PM, PM +H, PM + H_20_2 to be pleasing from 0-6hrs. However, RM+ H_20_2, PM, PM +H, continued to be pleasing to the 18th hour. Only PM + H_20_2 was found to be pleasing for up to 24 hours. This result showed that hydrogen peroxide is effective for sustaining the flavor of milk compared to honey for both raw and pasteurized milk. The result of this present study did not agree with the findings of Saha *et al* .,(2003) who reported that flavor of fresh milk treated with 0.03% H_20_2 was acceptable for up to 22 hours.

Treatment	0hr	6hr	12hr	18hr	24hr	30hr	Mean±SD
Flavour	***Quality***						
RM	PL	PL	SR	SR	BT	OF	-
RM+H	PL	PL	SS	SR	BT	OF	-
RM+H_2o_2	PL	PL	PL	PL	SS	SR	-
PM	PL	PL	PL	PL	SS	SR	-
PM+H	PL	PL	PL	PL	SS	SR	-
PM+ H_2o_2	PL	PL	PL	PL	PL	SS	-
Score							
RM	100.00	88.00	68.00	52.00	36.00	20.00	60.67±28.22
RM+H	100.00	100.00	80.00	64.00	48.00	24.00	69.33±26.22
RM+H_2O_2	100.00	100.00	100.00	92.00	80.00	64.00	89.33±24.01
PM	100.00	100.00	100.00	88.00	72.00	64.00	87.33±18.37
PM+H	100.00	100.00	100.00	88.00	76.00	64.00	88.10±21.89
PM+ H_2O_2	100.00	100.00	100.00	100.00	92.00	88.00	96.67±34.38

RM=Raw milk, H=Honey, PM= Pasteurized milk,PL=pleasing, ss= slightly sour, SR=sour,BT= Bitter, OF=off flavour, hr= hour

Table 3 : Flavor Quality and Score Control of Raw and Treated milk

From table 4, the texture score of RM decreased to 11.11% at the 12th hour probably due to the activities of microorganisms, it was possible that honey inhibited the growth of microorganisms in the raw milk and the score reduced to only 80% at the 12th hour. Green (1988) is of the opinion that sugar content of honey is exclusively responsible for its antibacterial effect. RM+ H_20_2 maintained 100% score only to the 12th hour and decreased to 73.00% at the 30th hour as compared to raw milk samples treated with honey which had a score of 100% only to the 6th hour and decreased to 11.11% at the 30th hour.

Parameters	0hr	6hr	12hr	18hr	24hr	30hr	MEAN ±SD
Texture Quality							
RM	FF	SF	CLT	CLT	CLT	CLT	___
RM+H	FF	FF	SF	CLT	CLT	CLT	___
RM+H_2O_2	FF	FF	FF	FF	SF	SF	___
PM	FF	FF	FF	SF	CLT	CLT	___
PM+H	FF	FF	FF	FF	CLT	CLT	___
PM+H_20_2	FF	FF	FF	FF	FF	FF	___
SCORECONTROL (%)							
RM	100.00	17.70	11.11	11.11	11.11	11.11	20.02
RM+H	100.00	100.00	80.00	11.11	11.11	11.11	52.22
RM+ H_20_2	100.00	100.00	100.00	93.30	80.00	73.00	91.05
PM	100.00	100.00	100.00	80.00	53.30	46.66	79.99
PM+H	100.00	100.00	100.00	86.66	53.30	46.66	81.10
PM+ H_20_2	100.00	100.00	100.00	100.00	100.00	100.00	100.00

RM=raw milk, PM=pasteurized milk,H=honey, hr=hour, CLT=clotted, FF=freeflowing SF=slightly flowing

Table 4: Texture Quality and Score Control of Raw and Pasteurized milk

The pH of untreated milk, pasteurized milk and milk treated with honey and hydrogen peroxide as presented on Table 5 had the normal pH as 6.5. The addition of either honey or hydrogen peroxide to raw or pasteurized milk did not alter the pH from 0-3hours. The pH dropped significantly with increased storage time for various milk samples. The drop in pH was very rapid in untreated milk followed by raw milk treated with honey, pasteurized milk treated with honey; raw milk treated with hydrogen peroxide and pasteurized milk treated with hydrogen peroxide. Significant differences ($p<0.05$) were observed for the acidity from the 3rd to the 30th hour for all the milk samples. The pH of fresh milk of 6.5 is comparable to the pH recorded by Olusola *et al* (2014). It is well known that the acidity in milk is developed due to the breakdown of milk sugar (lactose) in to lactic acid by the fermentative effect of acid producing bacteria when the storage time is lengthened.

pH Readings of Treated and Untreated Milk

Hours	RM	RM+H	RM+H_2O_2	PM	PM+H	PM+ H_2O_2
0	6.5±0.00	6.5±0.00	6.5±0.00	6.5±0.00	6.5±.0.00^{a}	6.5±.00
3	6.2±0.03^{b}	6.3±0.00^{b}	6.5±0.00^{a}	6.5±0.07^{ab}	6.5±0.07^{a}	6.5±0.00^{a}
6	6.1±0.07^{c}	6.2±0.07^{b}	6.5±0.07^{a}	6.3±0.00^{abc}	6.5±0.10^{a}	6.5±0.03^{a}
9	5.7±0.23^{c}	6.0±0.10^{abc}	6.3±0.00^{a}	6.3±0.07^{a}	6.3±0.17^{b}	6.5±0.10^{a}
12	5.0±0.13^{c}	5.7±0.23^{ab}	6.0±0.00^{b}	6.3±0.07^{a}	6.0±0.17^{b}	6.3±0.00^{a}
15	4.6±0.03^{c}	5.0±0.06^{d}	6.0±0.00^{b}	6.1±0.03^{a}	5.5±0.03^{c}	6.0±0.00^{a}
18	4.4±0.03^{d}	4.5±0.03^{d}	6.0±0.00^{a}	6.0±0.00^{b}	5.5±0.1^{c}	6.0±0.00^{a}
21	4.0±0.07^{d}	4.2±0.67^{d}	6.0±0.00^{a}	5.5±0.07^{b}	5.0±0.17^{c}	6.0±0.00^{a}
24	3.9±0.00^{d}	3.8±0.03^{d}	6.0±0.00^{a}	5.0±0.07^{b}	4.6±0.10^{c}	6.0±0.00^{a}
27	3.0±0.10^{c}	3.2±0.07^{d}	6.0±0.00^{a}	4.4±0.00^{c}	4.5±0.00^{c}	6.0±0.00^{a}
30	2.5±0.03^{d}	2.7±0.03^{d}	6.0±0.00^{a}	3.0±0.17^{c}	3.9±0.03^{d}	6.0±0.00^{a}

a, b, c, d=Means with different subscripts on the same row are significantly different ($p<0.05$) RM=Raw milk, H=Honey, PM= Pasteurized milk

Figure 5

5. Conclusion and Recommendations

From this study, it was observed that hydrogen peroxide was effective for controlling the flavor of milk compared with honey in both raw and pasteurized milk. Raw milk should be pasteurized using available materials at pasteurization time and temperature. Aside from microbial quality testing, drug residue and other tests, and identification of contaminants at species level should be conducted.

6. References

i. Coorevits, A, De Jonghe, V, Vandroemme, J. Reekmans, R, Heyrman, J, Messens, W,De Vos, P. and Heyndrick(2008). Conventional dairy farms. System. Appl. Microbiol.,41,121-125

ii. Griffiths, M.W./ Philips, J.D./ Muir, D.D. (1998).Thermostability of proteasese and lipases from a number of species of Food-borne diseases in France and in different industrialised countries. J. Food Microbiol., 67(2001)1–2, 1–17. , J.P.F./ Macdonald, A.M.C. Mycotoxins. Animal Feed Sci. Techn., 69, 155–166.

iii. Green, A,E(1998). Wound healing properties of honey.Journal of Medicine75-79

iv. Komorowski, E.S. and Maine R.(1992). The technology of dairy product,VCH publishers, Newyork)22-24

v. Rogelj, I. Mleko U,.(2003). In: Mikrobiologija živil živalskega izvora (Eds.: Bem, Z./ Adamič, J./ Žlender, B./ Smole Možina, R.K.). London and New Yersey, Applied Science Publishers, 1990, 41–108.

vi. Saha, B. K., Ali, M. Y., Chakraborty, M., Islam, Z., Hira, A. K. (2003). Study on the preservation of raw milk with hydrogen peroxide (H_2O_2) for rural dairy farmers. Pakistan Journal ofNutrition.2: 36–42.